Enzyme Wave

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Historic Villages of Shirakawa-go

The historic villages of Shirakawa-go (UNESCO World Heritage Site), located at Shirakawa in Gifu prefecture, are famous for their gassho-zukuri style houses.The gassho-zukuri style is characterized by a thatched and steeply slanting roof resembling two hands joined in prayer. This unique shape is said to have evolved in order to allow for the easier removal of snow from the roof in this heavy snowfall region. Moreover, all the house roofs face east-west, enabling them to receive direct sunlight evenly and to fend off the strong winds blowing down the north-south valleys.The beauty of these gassho-zukuri style houses reflects a rational and reasonable approach to architecture in which their function is well adapted to daily life and natural conditions in this region.

AMANO

It is known that there are a lot of food components of which chronic deficiency harms human health conditions. In Japan, the intake of some of these food components has unconsciously been decreased as a result of the westernization of dietary habits. Dietary fiber is one example of food components of which intake is decreased by such westernization.

Decreased dietary fiber intake, along with the increased dietary fat intake which is also a feature of the westernization of dietary habits, is thought to cause the rapidly increasing incidence of diseases more commonly found in western countries, such as obesity, diabetes mellitus, arteriosclerosis, cholelithiasis, breast cancer and colon cancer. A daily intake of 20 to 25 grams of dietary fiber is recommended in order to prevent these diseases, but the fact is that an enough amount of dietary fiber is not always taken.

Indigestible oligosaccharides are an alternative to dietary fiber and are believed to exert diverse actions that can help maintain good health conditions, including delayed digestion and absorption, inhibited absorption of cholesterol, and improved intestinal flora. Therefore, the widespread application of such indigestible oligosaccharides can be expected in areas such as the prevention of diabetes mellitus and cardiac diseases, the inhibition of colon cancer, and the treatment of inflammatory bowel disease.

However, it is not always easy to consume an enough amount of oligosaccharide in the form of a supplement in order to maintain good health conditions. We are therefore researching ways in which the intake of a small amount of oligosaccharide-producing enzymes as supplements, which can produce oligosaccharides from food components , may be substituted for the intake of the oligosaccharides themselves.

The latest research results are shown below.

The use of oligosaccharide-producing enzymes to produce oligosaccharides from ingested foods

Transglucosidase, an oligosaccharide-producing enzyme, produces indigestible oligosaccharides (e.g., isomaltose, isomaltotriose, panose) from digestible starch. Levansucrase, another oligosaccharide-producing enzyme, produces indigestible oligosaccharides (lactosucrose, levan) from lactose and sucrose.

Since these enzymes can maintain their enzymatic activity in an acid environment in the stomach, they are able to produce oligosaccharides from foods that are ingested orally. In fact, the in vivo production of oligosaccharides from ingested foods in rats has already been demonstrated experimentally.

In addition, animal studies have confirmed that administration of oligosaccharide-producing enzymes with feed is effective in preventing weight gain and improving intestinal flora. These findings are described in "Enzyme Wave Vol. 1."

Development of a dietary supplement enzyme for the prevention of diabetes mellitus -verification of effects in animal studies-

The development of dietary supplement enzymes is being advanced as part of a collaborative initiative involving Nagoya City University and Nippon Veterinary and Life Science University.

Professor Toshinori Sako, at Nippon Veterinary and Life Science University, investigated the effect of transglucosidase intake on blood glucose, insulin secretion and blood triglyceride in a study using diabetes model dogs and normal dogs.

In normal dogs to which transglucosidase was administered, even though postprandial glucose levels did not change, insulin secretion and the absorption of triglycerides were inhibited. The inhibition of insulin secretion observed in normal dogs is thought to have resulted from transglucosidase inhibiting the absorption of orally ingested carbohydrates. Moreover, the inhibited absorption of triglyceride is also thought to reflect the effect of oligosaccharide production.

In a single-dose study of transglucosidase in diabetes model dogs, inhibition of increase in postprandial glucose levels was observed. Moreover, in a two-week repeated-dose study, transglucosidase intake was shown to improve blood glucose control by decreasing blood fructosamine levels, an index of diabetes mellitus.

Development of a dietary supplement enzyme for the prevention of diabetes mellitus -Inhibition of postprandial glucose levels and insulin secretion in humans-

A clinical study of transglucosidase in healthy volunteers was conducted by Professor Takashi Joh and Doctor Makoto Sasaki of the Nagoya City University Graduate School of Medical Sciences. This was a randomized, placebo-controlled, three-way crossover trial involving 21 volunteers (17 males and 4 females, with a mean age of 48.3 years) in compliance with the WORLD MEDICAL ASSOCIATION DECLARATION OF HELSINKI Ethical Principles. The mean body mass index (BMI) of the volunteers was slightly high at 24.7 ± 3.1, suggesting a tendency towards obesity. However, the hemoglobin A1c was 5.4 ± 0.5% (4.6 to 6.4) and the fasting blood glucose was 95.3 ± 10.8 mg/dL (77 to 112), indicating that diabetic individuals had not been included as test subjects in this study.

After the volunteers were fed test meals with a total energy content of 522 kilocalories (protein, 14.4 g; fat, 2.1 g; carbohydrate, 111 g) and given transglucosidase (0 mg, 150 mg, or 300 mg) orally, their blood glucose and insulin levels were measured over time (at 0, 30, 60, 90, 120, 150, and 180 minutes postdose) and the effect of transglucosidase on postprandial glucose levels was evaluated. It was found that a postprandial increase in glucose levels tended to be inhibited in the enzyme-treated group (transglucosidase 150 mg or 300 mg) compared with the control group (transglucosidase 0 mg), although no effect on insulin secretion was observed (Figures 1 and 2).

Fig.1 Postprandial Blood Glucose Level

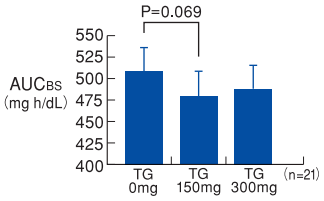
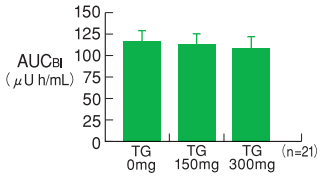


Fig.2 Postprandial Blood Insulin Level



However, in a sub-analysis of the group of subjects who were suspected of having impaired glucose tolerance because of a postprandial glucose level of 140 mg/dL or more when transglucosidase was not administered (N = 17), a postprandial increase in glucose levels was significantly inhibited in the transglucosidase-treated group compared with the control group and insulin secretion also tended to be inhibited (Figures 3 and 4). These results suggest that transglucosidase may be useful for preventing the development of diabetes mellitus.

Fig.3
Postprandial Blood Glucose Level
-Diabetic High-Risk Group (BS>140mg/dL)-

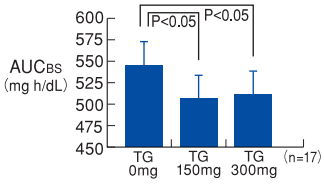
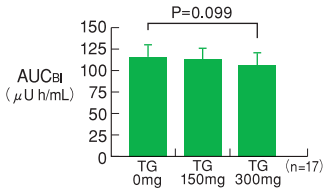


Fig.4
Postprandial Blood Insulin Level
-Diabetic High-Risk Group (BS >140mg/dL)-

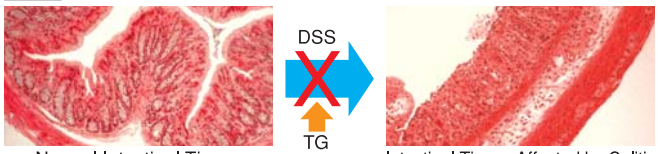


Development of a dietary supplement enzyme for the prevention of inflammatory bowel disease

In animal studies, the oral administration of transglucosidase was shown to provide a prebiotic effect, improving the intestinal flora. The ability of transglucosidase to reduce the symptoms of inflammatory bowel disease (IBD) by application of this prebiotic effect is being investigated by Professor Takashi Joh and Doctor Makoto Sasaki of the Nagoya City University Graduate School of Medical Sciences. Many cases of IBD have been reported in European countries and the U.S; the number of patients with this disease in Japan has also been growing rapidly in recent years, along with the westernization of dietary habits.

Dextran sodium sulfate (DSS)-treated mice were used as an IBD model animal. In mice treated with 3% DSS for five days, gastrointestinal bleeding and body weight loss were observed. Histological examination revealed inflammation of the intestinal mucosa layer as well as destruction of the mucosal layer and thickening of the muscular layer (Figure 5).

Fig.5 Prevention of IBD by transglucosidase (TG)



Administration of transglucosidase at 5 mg/kg or 15 mg/kg for two days before treatment with DSS resulted in the inhibition of destruction of the intestinal mucosal layer and the thickening of the muscular layer which were caused by inflammation. This effect can be attributed to the improved intestinal flora induced by oligosaccharides, which were produced from food by the transglucosidase.

Since a definitive treatment for IBD has not yet been established, it is necessary to first inhibit inflammation and then maintain the condition for a long period of time in order to prevent recurrence. Therefore, the development of foods or supplements designed to maintain and prevent recurrence can be expected. Since dietary restrictions are also required in order to prevent the recurrence of IBD, it is a concern that the patient's quality of life (QOL) could be diminished. Active ingredients produced from foods by transglucosidase supplements are therefore considered an effective means of improving the dietary aspects of the patient's QOL.

Future prospects

The current state of progress regarding the development of oligosaccharide-producing enzymes as dietary supplements to prevent diabetes mellitus and relieve the symptoms of inflammatory bowel disease has been outlined above.

In a large clinical study, an alpha-glucosidase inhibitor (acarbose) which is used for the treatment of diabetes mellitus has been shown to prevent the development of diabetes mellitus through its pharmacologic effect, suppressing carbohydration absorption. This finding suggests that controlled digestion and absorption is an essential factor in preventing the development of diabetes mellitus. This, in turn, suggests that our proposed method for the control of digestion and absorption using the oligosaccharides which are derived from ingested foods by oligosaccharide-producing enzymes has good prospects for preventing the development of diabetes mellitus. Previous studies have demonstrated that ingested foods are converted to oligosaccharides by oligosaccharide-producing enzymes within the gastrointestinal tract. Moreover, the results from these studies confirm the suppression of food absorption by oligosaccharides, based on suppressed blood glucose increase, insulin secretion, and weight gain. In particular, confirmation of the suppression of postprandial increase in blood glucose levels and insulin secretion in humans suggests the strong likelihood of being able to prevent the development of diabetes mellitus by using transglucosidase.

Furthermore, the administration of transglucosidase has not been associated with any adverse effect to date, and has not raised any potential safety concerns.

With regard to IBD, although animal studies are still underway, one study has already shown that the administration of transglucosidase suppresses the development of colitis in DSS-induced colitis mice, the model animal for IBD. The mechanism responsible for this suppression is now being investigated by researchers at Nagoya City University.

Many cases of IBD have been reported in Japan, and since the number of patients with this disease is expected to rise to levels comparable with those in European countries and the U.S. in the near future, it is a concern that many individual's QOL will be diminished because of dietary restrictions. The use of transglucosidase supplements is based on a new concept that regards oligosaccharides as an active ingredient produced from regular diets and which can be used to relieve the symptoms of IBD and prevent any recurrence of the disease. It is therefore expected that the use of these supplements will be able to contribute to the improvement of QOL, with regard to the diet of patients with IBD.

Another confirmed effect of the oral administration of transglucosidase is the suppression of triglyceride absorption. If these enzymes can also prevent hyperlipidemia, they could be expected to prevent not only hypertension (secondary to hyperlipidemia) but also cardiovascular diseases such as myocardial infarction and cerebral infarction.

Levansucrase, another oligosaccharide-producing enzyme, has also been shown to produce oligosaccharides within the gastrointestinal tract. A combination of transglucosidase and levansucrase should, therefore, be able to produce an even wider range of oligosaccharides and could be expected to help prevent the development of a variety of diseases that are becoming increasingly prevalent and to relieve the symptoms of these diseases.

Topics Nagoya COP 10；The 10th Conference of the Parties to the Convention on Biological Diversity

Today, the protection of the global environment is being taken more seriously as a problem that affects the entire world.

It has been decided that the 10th Conference of the Parties to the Convention on Biological Diversity (Conference of the Parties: COP 10), hosted by Japan, will be held between October 18 and 29, 2010, with the Nagoya Congress Center in Nagoya City as its main venue. The COP is an international conference at which various frameworks are developed by the contracting countries to the convention on biological diversity in order to accomplish the three established goals listed below, and to conserve and protect the biological diversity of every form of life on Earth, ranging from microorganisms to plants and animals.

Goals of the Convention on Biological Diversity

1) Conservation of biological diversity
2) Sustainable use of its components
3) Fair and equitable sharing of the benefits arising out of the utilization of genetic resources

"Biological diversity" means the presence of a variety of ecosystems and the variability within and between species, and is made up of the following three components: ecosystem diversity, species diversity, and genetic diversity.

Today, this biodiversity is threatened not only by the three dangers of "human-induced ecological disorder," "decreased attention paid to ecology by humans," and "disturbance of ecology by alien organisms, etc." but also by the fourth danger of "global warming." Every year, many organisms face the threat of extinction due to the ongoing destruction of their natural environment.

The Nagoya COP 10 will discuss the following:

1)The "Post-2010 Biodiversity Nagoya Target," following on from the 2010 target to "significantly reduce the rate of loss of biological diversity"
2)The "Nagoya Protocol" as an international framework regarding genetically modified organisms
3)The "Nagoya Regime" as an international framework regarding the access to and benefit-sharing of gene resources

In addition, the Nagoya COP 10 is expected to develop a "Nagoya Model for urbanization and biodiversity" as a regionally inspired outcome from the conference, and to provide the art of manufacturing learned from nature, as seen in fields such as biotechnology and biomimicry.

The enzyme industry, an industry based on the blessings of nature, is also paying attention to ecological initiatives such as these.

Topics Announcement of Completion of the Quality Assurance Center

The construction of the Quality Assurance Center, which carries out the key quality assurance activities of the Amano Enzyme Group, was completed in August 2008 within the site of the Nagoya Plant (located in Kita-nagoya City).

The quality assurance center was built in accordance with the concept of "a vibrant and comfortable work environment" and the need for "centralized quality assurance and control throughout the Amano Enzyme Group." This center houses both the quality assurance and quality control departments. The Quality Assurance Department is responsible for assuring product quality, providing quality information for customers and addressing various regulations by anticipating the actions of the regulatory authorities in individual countries. We are also a member of the Japan Food Additives Association and various other organizations (the Enzyme Technical Association (ETA), the Association of Manufacturers and Formulations of Enzyme Products (AMFEP), the Japan Bulk Pharmaceutical Manufacturers Association, the Osaka Pharmaceutical Manufacturers Association, and the Japan Parenteral Drug Association) and have exerted our efforts to contribute actively to the development of the enzyme industry.

The Quality Control Department is responsible for evaluating product quality using standard, internationally approved testing methods in accordance with various official compendiums such as the Japanese Pharmacopoeia, the Japanese Standards of Food Additives, the Food Chemicals Codex, and the FDA/BAM (Bacteriological Analytical Manual).

Testing parameters used are as follows:

- Enzyme activities (starch saccharifying activity, starch dextrinizing activity, protein digestive activity, fat digestive activity, etc.)
- General tests (identification, loss on drying, etc.)
- Purity tests (heavy metals limit test, arsenic limit test, lead limit test, etc.)
- Microbial limit tests (total viable aerobic count, coliforms, ***Escherichia coli***, etc.)

The Amano Enzyme Group assures our customers that we will continue to provide products, services and information, all of which are now controlled in the GMP-compliant laboratory in our Quality Assurance Center.

Description of the Quality Assurance Center
Building: Steel construction, two floors above ground, total floor area approximately 1,900 m^2
Floor plan
1st floor: Quality Assurance Department office, document storage room, meeting room, microorganism testing room
2nd floor: Testing room, Quality Control Department office

Topics Toward the Establishment of a Historical Archive of Enzymology

Traditional Japanese foods such as sake (rice wine), miso (soy-bean paste) and shōyu (soy sauce), all of which are produced by Koji fermentation (the utilization of ***Aspergillus oryzae***), are just some examples of the way in which enzymes have played a crucial role in the history of world-class biotechnology in Japan. Amano Enzyme Inc. is based on the tradition culture of Japan which can best be described as "living in harmony with nature and conserving natural resources" and has involved in the utilization of the enzymes generated by various microorganisms, such as ***Aspergillus oryzae***, and plants and animals for many years.

Looking back on the history of the discovery and utilization of enzymes which seem to have originated in Japan, contributions are achieved by many universities and companies involved. Unfortunately, however, we have had no place to compile and publicize their accomplishments. In accordance with our corporate philosophy that "we create new value and contribute to society through the application of our enzymes," Amano Enzyme Inc. has therefore decided to collect materials including books related to the history of the discovery and utilization of enzymes and to set up a "Historical Archive of Enzymology" in order to make that information public.

In order to establish the enzyme library in 2010, a group comprised mainly of retirees from our company has already started collecting relevant data. We have also been informed that many universities and companies will supply invaluable books and data relating to enzymes. We are deeply indebted to many universities and companies for their continued support in setting up the Historical Archive of Enzymology.

We are now preparing to set up the Historical Archive of Enzymology, hoping that more and more people will gain a better understanding of enzymes and that we will thereby be able to contribute to the promotion and further utilization of enzymes.

We thank you for your assistance and co-operation in our project.

About the Historical Archive of Enzymology

1. Concept

To contribute to the public understanding of the history of the discovery and utilization of enzymes

2. Details

1) Exhibition of historical materials including books related to enzymes and the utilization of enzymes
2) Display of invaluable data and panels showing the historical development of enzymes and the utilization of enzymes
3) Setting up display corners to support and popularize the understanding of enzymes

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